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Urochordamines A and B: Larval Settlement/Metamorphosis-Promoting, Pteridine-Containing Physostigmine Alkaloids from the Tunicate *Ciona savignyi*

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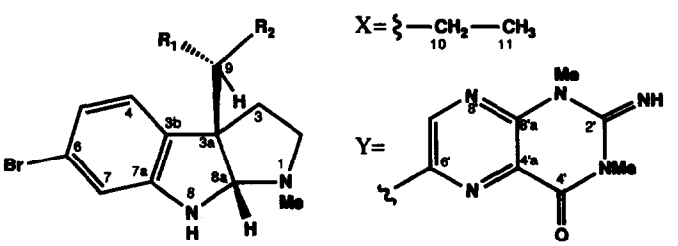
Abstract: Two pteridine-containing bromophysostigmines which promote settlement and metamorphosis of the tunicate *Ciona savignyi* larvae have been isolated from the tunic (outer body) of the adult tunicate *C. savignyi*. The structures were determined by extensive spectral analysis.

Larvae of marine organisms are believed to initiate settlement, followed by metamorphosis, upon reception of chemical cues.[1] Although this phenomenon is known for a wide range of marine organisms, those with identified chemical cues are quite few, e.g. δ-tocopherols[2] for the hydrid *Coryne uchidai*, fatty acids[3] for the annelid *Phramatopoma californica*, and jacaranone[4] for the scallop *Pecten maximus*. In the course of our studies of mechanisms for marine biofouling, we found that a lipophilic extract of the tunic of the tunicate *Ciona savignyi* promoted settlement and metamorphosis of its larvae.[5] Bioassay-guided isolation afforded an active compound named urochordamine A, and its stereoisomer, urochordamine B, which are unusual bromophysostigmine alkaloids encompassing a pteridine unit. We describe the isolation and structure elucidation of these compounds.

The tunic was dissected from the tunicate *Ciona savignyi*, collected off Asamushi, 600 km northeast of Tokyo, in June, 1992, and extracted with MeOH. The concentrated residue was extracted with Et_2O, then with *n*-BuOH. The *n*-BuOH layer, which promoted settlement and metamorphosis of the tadpole larvae of *C. savignyi*, was fractionated by ODS flash (aq. MeOH) and silica gel column chromatography ($CHCl_3$/MeOH), followed by normal phase HPLC ($CHCl_3$/MeOH/H_2O) to yield an active principle, urochordamine A (**1**).[6]



Urochordamine A (**1**): R_1=X, R_2=Y
Urochordamine B (**2**): R_1=Y, R_2=X

Fig. 1 Structures of Urochordamines A (**1**) and B (**2**)

The less active compound, urochordamine B (**2**),[7] was also isolated from the slightly less polar fraction (yields: **1**, 3.7 x 10^{-4}; **2**, 1.2 x 10^{-4} % wet weight). The ^{1}H NMR spectra ($CDCl_3$) of both compounds showed that their structures were similar. However, two aromatic protons in **1** overlapped; we therefore elucidated structure of **2** first.

Urochordamine B (**2**)[7] had a molecular formula of $C_{22}H_{26}BrN_7O$ as determined by the HRFAB mass spectrum [*m/z* 486.1448, Δ +0.8 mmu, $(M+H)^+$ for $C_{22}H_{27}{}^{81}BrN_7O$]. The ^{1}H NMR data together with COSY experiments revealed the presence of a 1, 2, 4-trisubstituted benzene ring [δ 6.49 (1 H, d, *J*=1.5 Hz, 7-H), 6.70 (1 H, dd, *J*=8.0 and 1.5 Hz, 5-H), and 6.90 (1 H, d, *J*=8.0 Hz, 4-H)], an ethylene [δ 1.84 (3α-H) and 2.36 (3β-H); 2.48 (2α-H) and 2.66 (2β-H)], an *N*-methyl at δ 2.41, and an exchangeable proton at δ 4.33 coupled to a methine at δ 5.09. Interpretation of 2D NMR spectra including HMBC data (Table 1) led to 3a-substituted 1-methyl-6-bromophysostigmine structure, which was also supported by the UV (252 nm) and IR spectra (3300 and 1620 cm^{-1}) as well as by FABMS fragment ions at *m/z* 252/250 (intensity, *ca.* 1:1). Moreover, the ^{13}C NMR data were superimposable on those reported for the relevant portion of dihydroflustramine C.[8]

The remaining portion was composed of $C_{11}H_{14}N_5O$, indicating 7 degrees of unsaturation. The presence of an *n*-propyl group was readily derived from the COSY crosspeaks [δ 0.70 (3 H, t, *J*=7.4 Hz, 11-

Table 1. ^{1}H and ^{13}C NMR Data of Urochordamine B (**2**) in $CDCl_3$

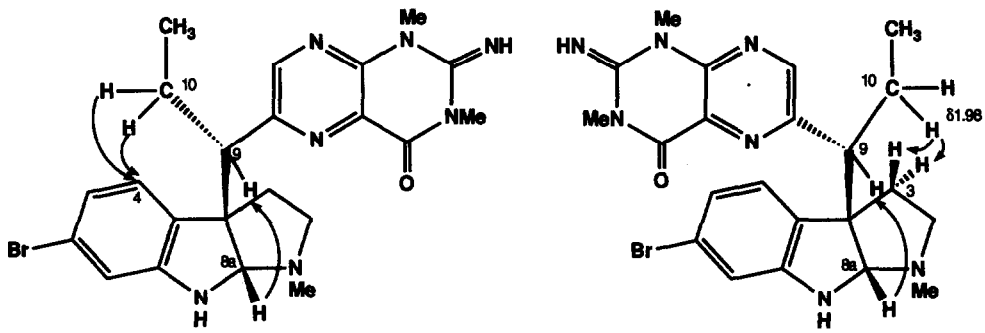
atom	^{13}C mult	^{1}H mult	*J* (Hz)	HMBC correlaions
2	51.8 t	α 2.48 td	8.8, 5.9	1-Me, C3, C3a, C8a
		β 2.66 br.s		C3, C3a, C8a
3	37.7 t	α 1.84 ddd	12, 5.9, 3.9	C2, C3a, C3b, C8
		β 2.36 m		C2, C3a, C3b, C9
3a	60.9 s			
3b	133.6 s			
4	125.1 d	6.90 d	8.0	C3a, C6, C7a
5	121.4 d	6.70 dd	8.0, 1.5	C3b, C6, C7
6	121.3 s			
7	112.4 d	6.49 d	1.5	C3b, C6
7a	152.3 s			
8a	84.7 d	5.09 s		1-Me, C2, C3, C3b, C7a, C9
9	53.2 d	3.08 dd	11, 2.8	C3, C3a, C3b, C8a, C10, C11, C6', C7'
10	22.8 t	α1.88 qdd	7.4, 14, 11	C9, C6'
		β1.98 qdd	7.4, 14, 2.8	C11, C6'
11	12.5 q	0.70 t	7.4	C9, C10
2'	150.2 s			
4'	159.3 s			
4'a	126.1 s			
6'	151.5 s			
7'	147.6 d	8.02 s		C6', C8'a
8'a	147.0 s			
8-NH		4.33 br.s		
1-Me	36.6 q	2.41 s		C2, C8a
1'-Me	29.7 q	3.61 s		C2', C8'a
3'-Me	29.5 q	3.51 s		C2', C4'

H_3), 1.88 (1 H, qdd, J=7.4, 14, and 11 Hz, 10α-H), 1.98 (1 H, qdd, J=7.4, 14, and 2.8 Hz, 10β-H), and 3.08 (1 H, dd, J=11 and 2.8 Hz, 9-H)]; this proton at δ 3.08 showed HMBC crosspeaks not only with C3a (δ 60.9), C3b (δ 133.6), C8a (δ 84.7), and C3 (δ 37.7) on the physostigmine nucleus, but also with deshieled carbons at δ 147.6 (C7') and 151.5 (C6'), thus revealing connectivities of C3a-C9-C6'-C7'. HMBC crosspeaks [δ 3.61(3 H, s, 1'-Me)/δ 147.0 (s, C8'a) and 150.2 (s, C2'); δ 3.51 (3 H, s, 3'-Me)/δ 150.2 (s, C2') and 159.3 (s, C4'); δ 8.02 (1 H, s, 7'-H)/δ 147.0 (s, C8'a) and 151.5 (s, C6')] and the ^{13}C NMR chemical shifts [δ 151.5 (C6'), 147.6 (C7'), and 147.0 (C8'a)] led to the structural unit, -N(5')-C(6')-C(7')H-N(8')-C(8'a)-N(1')Me-C(2')-N(3')Me-C(4')-. With 7 degrees of unsaturation, a pteridine nucleus was consistent with these data, which was also supported by a UV absorption at 314 (log ε 3.54) and 355.5 (3.68) nm. The connection of the pteridine and the physostigmine units via C9 methine of the *n*-propyl group was straightfoward by HMBC crosspeaks (Table 1). Thus, the gross structure **2** was completed.

Urochordamine A (**1**)[6] has the same molecular formula as **2**. ^1H and ^{13}C NMR spectra suggested that **2** was a congener of **1**. Analyses of 2D NMR data disclosed that **1** had the same structure as **2** except for stereochemistry.

Relative stereochemistry of **1** and **2** was deduced by NOESY experiments. The crosspeaks between 8a-H and 9-H for both **1** and **2** secured *cis* relationship of the fused pyrroles. Both C10 methylene protons showed NOE's with the 4-H proton on the benzene ring in **1**, whereas one of the C10 methylene protons (δ 1.98) in **2** had NOE's with the C3 methylene protons, thereby suggesting that the stereochemistry at C9 was opposite in **1** and **2**. Thus, the relative stereochemistry for urochordamine A and B is as shown in Fig. 2.

Urochordamine A (**1**) promoted larvae settlement and metamorphosis in *C. savignyi* at a concentration of 2 ng/mL; all larvae treated with **1** completed settlement and metamorphosis by the time 50 % of larvae in a control group had settled.[9] Interestingly, urochordamine B (**2**) had no activity at the same concentration, thus suggesting the importance of stereochemistry at C9. It should be noted that **1** and **2** were not detected in *C. savignyi* collected at the same place in October. Therefore, **1** and **2** may be produced only during spawning season, which is reasonable considering the function. Moreover, the colonial tunicate *Botrylloides* sp. collected in the Gulf of Sagami in June contained both compounds (yield: **1**, 6.8 x 10^{-4} ; **2**, 6.0 x 10^{-4} %



Urochordamine A (**1**) Urochordamine B (**2**)

Fig. 2 NOE Observed for Urochordamines A (**1**) and B (**2**)

wet weight), which may indicate that tunicates generally produce these promoters.[10] Bromophysostigmines have been known from the marine bryozoan *Flustra foliacea*,[8] while C6-substituted pteridines were reported from the marine sponge *Leucetta microraphis*,[11] the anthozoan *Astroides calycularis*,[12] and the polychaete *Odontosyllis undecimdonta*,[13] which may suggest that microorganisms play a role in the biosynthesis of urochordamines.

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5. The tadpole larva attatches by its head to the surface of a dish (settlement) after swimming for a species-specific period, and its tail is gradually absorbed into the adult (metamorphosis; during which some of its chordate characteristics disappear). The details of the screening will be reported elsewhere.
6. Urochordamine A (**1**): $[\alpha]_D$ +11.7° (*c*, 0.263, $CHCl_3$). IR ν_{max} (KBr) 3300 (br.), 1690, 1620, 1540, 1490, 1450, and 750 cm^{-1}. UV λ_{max} (MeOH) 211.5 (log ε 4.52), 253 (4.30), 312 (3.62), and 357 (3.74) nm. ^1H NMR ($CDCl_3$) δ 0.66 (3 H, t, *J*=7.4 Hz, 11-H3), 1.82 (1 H, ddq, *J*=14, 2.8, and 7.4 Hz, 10α-H), 1.93 (1 H, tq, *J*=14 and 7.4 Hz, 10β-H), 2.07 (1 H, dt, *J*=13 and 6.4 Hz, 3α-H), 2.19 (1 H, dt, *J*=13 and 6.4 Hz, 3β-H), 2.32 (3 H, s, 1-Me), 2.45 (1 H, dt, *J*=9.3 and 6.4 Hz, 2β-H), 2.59 (1 H, dt, *J*=9.3 and 6.4 Hz, 2α-H), 3.15 (1 H, dd, *J*=12 and 2.8 Hz, 9-H), 3.53 (3 H, s, 3'-Me), 3.65 (3 H, s, 1'-Me), 4.20 (1 H, br.s, 8-H), 4.62 (1 H, br.s, 8a-H), 6.61 (1 H, s, 7-H), 6.84 (2 H, 4 and 5-H_2), and 7.82 (1 H, s, 7'-H). ^{13}C NMR ($CDCl_3$) δ 12.5 (q, C11), 23.5 (t, C10), 29.6 (q, N3'-Me), 29.8 (q, N1'-Me), 37.1 (t, C3), 37.5 (q, N1-Me), 50.0 (d, C9), 52.5 (t, C2), 60.9 (s, C3a), 85.6 (d, C8a), 112.3 (d, C7), 121.5 (d, C5), 122.0 (s, C6), 125.3 (d, C4), 125.8 (s, C4'a), 132.1 (s, C3b), 147.0 (d, C7'), 147.5 (s, C8'a), 150.3 (s, C2'), 151.1 (s, C6'), 151.7 (s, C7a), and 159.4 (s, C4'). The cross peaks observed in the COLOC spectrum: 4- and 5-Hs/C3b, C6, C7, and C7a; 7-H/C3b, C5, C6, and C7a; 8a-H/C7a; 9-H/C3a, C10, and C6'; 7'-H/C6' and C8'a; 1-Me/C2 and C8a; 1'-Me/C2' and C8'a; 3'-Me/C2' and C4'. FABMS (positive, glycerol matrix) *m/z* 486/484 $(M+H)^+$, 406 $(M-Br+H)^+$, 252/250 $(C_{11}H_{12}BrN_2-H)^+$, 232 $(C_{11}H_{14}ON_5)^+$, and 218 $(C_{10}H_{12}ON_5)^+$. HRFABMS *m/z* 486.1441 (calcd for $C_{22}H_{27}{}^{81}BrN_7O$, Δ +0.1 mmu). The name urochordamine was coined after the subphylum Urochordata to which *C. savignyi* belongs.
7. Urochordamine B (**2**): $[\alpha]_D$ -36.6° (*c*, 0.174, $CHCl_3$). IR ν_{max} (KBr) 3300 (br.), 1690, 1620, 1540, 1490, 1460, and 750 nm^{-1}. UV λ_{max} (MeOH) 210 (log ε 4.46), 252 (4.23), 314 (3.54), and 355.5 (3.68) nm. FABMS (positive, glycerol matrix) *m/z* 486/484 $(M+H)^+$, 406 $(M-Br+H)^+$, 252/250 $(C_{11}H_{12}BrN_2-H)^+$, 232 $(C_{11}H_{14}ON_5)^+$, and 218 $(C_{10}H_{12}ON_5)^+$. HRFABMS *m/z* 486.1448 (calcd for $C_{22}H_{27}{}^{81}BrN_7O$, Δ +0.8 mmu).
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9. Urochordamines (**1** and **2**) showed antibacterial activity against a gram positive bacterium, *Bacillus marinus* ATCC 29841; 22 mm of inhibitory zone for **1** and 18 mm for **2** at 0.2 mg/disk (0.8 mmϕ). However, both compounds were inactive against the fungus, *Mortierella ramaniana*, or gram negative bacteria, *Pseudomonas nautica* IAM 12929 or *Flavobacterium marinotipycum* ATCC 19260 at the same doses.
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